

## Amendments to the Specification

Please replace page 40 of the specification as follows:

Calix Limos (M/L)													
Primary Structure	H <sub>2</sub> O		H <sub>2</sub> O		H <sub>2</sub> O		H <sub>2</sub> O		H <sub>2</sub> O		H <sub>2</sub> O		H <sub>2</sub> O
	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	
70	100	100	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100	100	100
20	100	100	100	100	100	100	100	100	100	100	100	100	100

*Antimicrobial activity:* On solid medium

*Bacillus subtilis.* 10 $\mu$ g/disk (6mm diameter): 10 mm inhibition zone

*Spectroscopic data:*

HRFABMS  $m/z$  509.275351 [M-H<sub>2</sub>O+H]<sup>+</sup> (calcd for C<sub>28</sub>H<sub>37</sub>N<sub>4</sub>O<sub>5</sub> 509.276396  $\Delta$  1.0 mmu); LRFABMS using m-NBA as matrix  $m/z$  (rel intensity) 509 [M-H<sub>2</sub>O+H]<sup>+</sup> (5), 460 (2.7), 391 (3).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d,  $J$  = 2.0 Hz, H-21), 4.62 (dd,  $J$  = 8.4, 3.7 Hz, H-1), 3.98 (bd,  $J$  = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d,  $J$  = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d,  $J$  = 7.4 Hz, H-26);

<sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-cap	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-22B	GI50	4.58E-06	3.08E-07	8.49E-07	3.02E-06	8.24E-07	5.20E-07	4.71E-06	1.13E-07	4.77E-06	1.01E-06	2.54E-06	6.95E-06	7.61E-07	4.65E-07
	TGI	8.62E-06	6.08E-07	2.30E-06	7.04E-06	2.28E-06	9.99E-07	8.83E-06	4.67E-07	1.17E-05	2.75E-06	6.84E-06	1.90E-05	1.83E-06	9.32E-07
	LC50	1.62E-05	1.20E-06	1.21E-05	1.65E-05	8.85E-06	2.01E-06	1.66E-05	1.84E-06	>1.90E-05	1.86E-05	1.84E-05	>1.90E-05	7.42E-06	1.86E-06

*Antimicrobial activity:* On solid medium

*Bacillus subtilis.* 10µg/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

HRFABMS  $m/z$  509.275351  $[M-H_2O+H]^+$  (calcd for  $C_{28}H_{37}N_4O_5$  509.276396  $\Delta$  1.0 mmu);

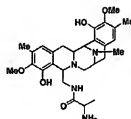
LRFABMS using m-NBA as matrix  $m/z$  (rel intensity) 509  $[M-H_2O+H]^+$  (5), 460 (2.7), 391 (3).

$^1H$  NMR ( $CD_3OD$ , 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d,  $J$  = 2.0 Hz, H-21), 4.62 (dd,  $J$  = 8.4, 3.7 Hz, H-1), 3.98 (bd,  $J$  = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d,  $J$  = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d,  $J$  = 7.4 Hz, H-26);

$^{13}C$  NMR ( $CD_3OD$ , 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Please replace page 41 of the specification as follows:

# **COMPOUND P-22A**



## **Strain:**

The same as for P-22B

## **Fermentation conditions:**

The same as for P-22B

## **Isolation:**

The same as for P-22B

## **Biological activities of safranal P-22A**

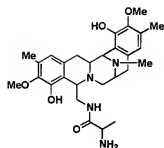
### Antitumor activities

Strain	Cells Lines (H-1)															
	HT-29	Hep-2	Hep-3	Hep-4	Hep-5	Hep-6	Hep-7	Hep-8	Hep-9	Hep-10	Hep-11	Hep-12	Hep-13	Hep-14	Hep-15	Hep-16
P-22A	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

### Antimicrobial activity: On solid medium

*Bacillus subtilis*, 10µg/disk (6mm diameter): NO ACTIVE

## COMPOUND P-22A



Strain:

The same as for P-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

Biological activities of safracin P-22A

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon		Cervix		
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HIT9	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-22A	GISO	>1.96E-05	4.19E-06	7.74E-06	1.30E-05	1.27E-05	5.93E-06	>1.96E-05	3.15E-06	>1.96E-05	1.26E-05	>1.96E-05	>1.96E-05	8.75E-06	7.66E-06
	TGI	>1.96E-05	9.26E-06	1.96E-05	>1.96E-05	>1.96E-05	1.33E-05	>1.96E-05	7.93E-06	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.96E-05
	LC50	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05

Antimicrobial activity: On solid medium*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

Please replace page 43 of the specification as follows:

*Biological activities of safracin P-19B*

Antitumor activities

Primary Screening	Cells Lines (Mol.)															
	Panc43	HT29	HepG2	MDA-MB-231	SK-N-SH	SK-N-BE	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH	SK-N-SH
HT29	660	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08
MDA-MB-231	100	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08	1.0E-08

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

Spectroscopic data:

HRFABMS  $m/z$  495.260410  $[M-H_2O+H]^+$  (calcd for  $C_{27}H_{35}N_4O_5$  495.260746  $\Delta$  0.3 mmu); LRFABMS using m-NBA as matrix  $m/z$  (rel intensity) 495  $[M-H_2O+H]^+$  (13), 460 (3), 391 (2);  $^1H$  NMR ( $CD_3OD$ , 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d,  $J = 7.1$  Hz, H-26);

## Biological activities of safracin P-19B

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-AFL
Safracin  P-19B	GI50	1.70E-05	3.90E-06	5.42E-06	8.74E-06	7.08E-06	7.90E-06	>1.95E-05	2.38E-06	1.81E-05	1.55E-05	>1.95E-05	1.44E-05	6.73E-06	4.80E-06
	TGI	>1.95E-05	8.06E-06	1.48E-05	>1.95E-05	1.92E-05	>1.95E-05	>1.95E-05	5.77E-06	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.61E-05	1.00E-05
	LC50	>1.95E-05	1.67E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.40E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.95E-05

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

Spectroscopic data:

HRFABMS  $m/z$  495.260410  $[M-H_2O+H]^+$  (calcd for  $C_{27}H_{35}N_4O_5$ ; 495.260746  $\Delta$  0.3 mmu);  
 LRFABMS using m-NBA as matrix  $m/z$  (rel intensity) 495  $[M-H_2O+H]^+$  (13), 460 (3), 391 (2);  
 $^1H$  NMR ( $CD_3OD$ , 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe),  
 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d,  $J = 7.1$  Hz, H-26);

Please replace page 46 of the specification as follows:

Cells Lines (Mol/L)													
Primary Structure	D. Protein		Oxy		Bovine		Human		MST		L. Proteins		pH
	1	2	3	4	5	6	7	8	9	10	11	12	
P4-Resonance de la C-15	CD	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03
	TI	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03
	LD	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03
Secondary Evaluation (Mol/L)													
Secondary Structure	Secondary Structure				Secondary Structure				Secondary Structure				pH
	1	2	3	4	5	6	7	8	9	10	11	12	
P4-Resonance de la C-15	CD	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03
	TI	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03
	LD	1.52E-03	1.51E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03	1.52E-03

**Antimicrobial activity:** On solid medium

*Bacillus subtilis*. 10 $\mu$ g/disk (6mm diameter): Inhibition zone: 15 mm diameter

#### Spectroscopic data

ESMS:  $m/z$  509 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d,  $J$  = 7.2 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon		Cervix		
		DU-145	LN-cap	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANCI	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin D	GI50	5.22E-06	1.54E-06	2.68E-06	1.33E-06	4.71E-06	3.51E-06	6.04E-06	6.04E-07	4.77E-06	4.33E-06	6.99E-06	4.75E-06	3.76E-06	2.28E-06
	TGI	9.99E-06	4.12E-06	6.02E-06	3.34E-06	7.82E-06	6.21E-06	1.07E-05	1.16E-06	1.10E-05	1.79E-05	1.82E-05	8.85E-06	6.68E-06	5.24E-06
	LC50	1.90E-05	9.78E-06	1.35E-05	9.15E-06	1.30E-05	1.10E-05	1.88E-05	3.78E-06	>1.90E-05	>1.90E-05	>1.90E-05	1.65E-05	1.19E-05	1.21E-05

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin D	IC50	1.90E-05	1.52E-05	3.80E-06	2.85E-06	6.65E-06

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter). Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS:  $m/z$  509  $[M-H_2O+H]^+$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d,  $J = 7.2$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.



Please replace page 48 of the specification as follows:

Cells Lines (Molt)														
Primary Generation	Cell Number		Day	Pass	Alkaline Phosphate	pH	pH	pH	pH	pH	pH	pH	pH	pH
	10 <sup>4</sup>	10 <sup>5</sup>												
PK-15 (Molt)	100	1000	10000	100000	1000000	10000000	100000000	1000000000	10000000000	100000000000	1000000000000	10000000000000	100000000000000	1000000000000000
PK-15 (Molt)	100	1000	10000	100000	1000000	10000000	100000000	1000000000	10000000000	100000000000	1000000000000	10000000000000	100000000000000	1000000000000000
PK-15 (Molt)	100	1000	10000	100000	1000000	10000000	100000000	1000000000	10000000000	100000000000	1000000000000	10000000000000	100000000000000	1000000000000000

Secondary Evaluation (Molt)														
Primary Generation	Cell Number		Day	Pass	Alkaline Phosphate	pH	pH	pH	pH	pH	pH	pH	pH	pH
	10 <sup>4</sup>	10 <sup>5</sup>												
PK-15 (Molt)	100	1000	10000	100000	1000000	10000000	100000000	1000000000	10000000000	100000000000	1000000000000	10000000000000	100000000000000	1000000000000000

**Antimicrobial activity:** On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

#### Spectroscopic data

ESMS:  $m/z$  511 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d,  $J = 6.6$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-cnP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin E	GI50	8.34E-06	3.86E-06	4.50E-06	4.54E-06	5.05E-06	3.94E-06	1.96E-05	4.25E-06	6.05E-06	7.89E-06	7.15E-06	5.07E-06	4.15E-06	4.03E-06
	TGI	1.96E-05	7.70E-06	8.85E-06	8.25E-06	9.24E-06	6.93E-06	>1.96E-05	8.21E-06	1.47E-05	1.96E-05	>1.96E-05	9.44E-06	7.29E-06	7.25E-06
	LC50	>1.96E-05	1.54E-05	1.74E-05	1.49E-05	1.70E-05	1.22E-05	>1.96E-05	1.59E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.75E-05	1.28E-05	1.30E-05

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin E	IC50			1.57E-05	>1.96E-05	

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS:  $m/z$  511  $[M+H]^+$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d,  $J = 6.6$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Please replace page 52 of the specification as follows:

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

#### Biological activities of safracin B (OEt)

##### Antitumor activities

Cells Lines (MOL)																
Primary Structure	P1446		P1447		P1448		P1449		P1450		P1451		P1452		P1453	
	1446	1447	1448	1449	1450	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460	1461
Safracin B	0.01	0.0150	0.0150	0.0200	0.0250	0.0300	0.0350	0.0400	0.0450	0.0500	0.0550	0.0600	0.0650	0.0700	0.0750	0.0800
TO	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
SAOEt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Secondary Evaluation (MOL)																
Secondary Structure	P1446				P1447				P1448				P1449			
	1446	1447	1448	1449	1450	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460	
Secondary Structure	0.01	0.0150	0.0150	0.0200	0.0250	0.0300	0.0350	0.0400	0.0450	0.0500	0.0550	0.0600	0.0650	0.0700	0.0800	

**Antimicrobial activity:** On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

##### Spectroscopic data:

ESMS: m/z 551 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

#### Biological activities of safracin B (OEt)

##### Antitumor activities

Cells Lines (Mol/L)																
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix		
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-AFL	
Safracin B (OEt)	G150	4.01E-07	4.84E-08	4.06E-08	6.82E-07	4.82E-08	1.69E-07	5.01E-07	3.97E-08	6.49E-07	2.44E-07	4.43E-07	2.09E-06	8.92E-08	7.70E-08	
	TGI	1.01E-06	>1.76E-05	9.97E-08	1.19E-06	1.16E-07	4.40E-07	1.16E-06	1.08E-07	2.06E-06	1.39E-06	1.09E-06	9.88E-06	3.15E-07	2.74E-07	
	LC50	1.60E-05	8.28E-07	4.27E-06	6.37E-06	1.02E-06	1.13E-06	5.66E-06	3.69E-06	1.35E-05	>1.76E-05	>1.76E-05	>1.76E-05	1.35E-06	9.76E-07	
Secondary Evaluation (Mol/L)																
Secondary Screening		Macromolecules Synthesis								Apoptosis			DNA Binding			
		PROTEIN				DNA		RNA		NUCLEOSOMES			GEL			
Safracin B (OEt)		IC50		>1.76E-05				1.76E-06		1.76E-07		5.28E-08			1.76E-05	

##### Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

##### Spectroscopic data:

ESMS: m/z 551 [M-H<sub>2</sub>O+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

Please replace page 54 of the specification as follows:

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

**Biological activities of safracin A (OEt):**

**Antitumor activities**

Cells Lines (MOL)															
Primary Screening	P. Fuchs				Orai				Hep-2				MDA-MB-231		
	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05
Safracin A (OEt)	0.01	1.0E-03	1.0E-07		1.0E-07	2.0E-05	1.0E-07	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05
10-0002	0.01	1.0E-03	1.0E-07		1.0E-07	1.0E-05	1.0E-07	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05
10-0002	0.01	1.0E-03	1.0E-07		1.0E-07	1.0E-05	1.0E-07	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05	1.0E-07	1.0E-05

Secondary Evaluation (MOL)																
Secondary Screening	MDA-MB-231				Hep-2				MDA-MB-231				MDA-MB-231			
	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05	
10-0002	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05	0.1	0.01	0.02	0.05	

**Antimicrobial activity:** On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 10 mm inhibition zone

**Spectroscopic data:**

ESMS: m/z 553 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

#### Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon		Cervix		
		DU-145	LN-CaP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HIT9	LOVO	LOVO-DOX	HELA	HELA-AFL
Safracin A (OEt)	GI50	2.64E-06	3.78E-07	4.92E-07	2.01E-06	5.55E-07	7.96E-07	4.00E-06	3.11E-07	3.06E-06	1.97E-06	2.03E-06	5.72E-06	1.02E-06	7.64E-07
	TGI	5.39E-06	7.42E-07	9.28E-07	5.10E-06	1.16E-06	1.90E-06	7.17E-06	6.86E-07	5.83E-06	4.41E-06	4.41E-06	9.84E-06	2.91E-06	2.32E-06
	LC50	1.10E-05	1.45E-06	1.76E-06	1.30E-05	5.57E-06	5.77E-06	1.28E-05	1.51E-06	1.11E-05	9.88E-06	9.88E-06	1.69E-05	7.85E-06	6.69E-06

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin A (OEt)	IC50			6.33E-06	1.81E-06	

Antimicrobial activity: On solid medium

*Bacillus subtilis*. 10µg/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)